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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a r quest f r filing a PRO[illegible]T under 37 CFR 1.53(c).

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☑ *Additional inventors are being named on the* 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Directional labeling of signal and affinity moieties on microparticles

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 3
☐ Drawing(s) *Number of Sheets*
☐ Application Data Sheet. See 37 CFR 1.76
☐ CD(s), Number
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Respectfully submitted,

SIGNATURE _____

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REGISTRATION NO. *(if appropriate)*

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Additional Page

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Directional Labeling of Signal and Affinity Moieties on Microparticles

This invention relates to a method or methods for labeling microparticles with signal generating moiety and analyte affinity moiety in a directional fashion. A variety of micro particles such as nano particles, micro spheres, micro beads and etc. can be employed in the invention. The purpose of this invention is to directionally label microparticles with both signal moiety and analyte affinity moiety on particle surface thereby minimizing potential interference of these two moieties for sensitive detection of an analyte. It may also and increase number of signal groups on the particle.

Microparticles may contain numerous functional chemical groups such as primary amine on their surface, which permits labeling of desired affinity groups for analyte capture. When both signal moiety (e.g., fluorescent compounds) and affinity moiety are present on particle surface or when the affinity group is on the surface while the signal molecules are encapsulated in the particles, microparticles may be used for detection of specific analytes. Microparticles are immobilized to a solid phase (e.g., magnetic particles) through the binding of specific analytes in the sample. After removal of unbound microparticles, the signal moiety /molecules are detected.

It is understood that microparticles that are applicable to this invention include, but are not limited to, microspheres, nanoparticles, liposome or the like, or other aggregates with appropriate surface functional groups.

Direct or indirect (e.g., through a carrier such as a polymer) labeling of signal compounds and affinity groups on to the particles can be applied. In both cases, however, the signal compounds may physically hinder the binding of affinity groups with analytes thereby reducing the binding efficiency of the affinity groups for the analytes.

In our invention, microparticles are directionally labeled with the affinity groups and signal compounds with the affinity groups present in the outermost layer while the signal compounds in a layer beneath the affinity group layer. An example is illustrated in Fig. 1. This layered or directional labeling method minimizes physical hindrance of the signal compounds with affinity binding thereby improving affinity binding efficiency. It may provide more signal molecules per particle for higher sensitivity.

The affinity group can be any chemical functionality with specific affinity for an analyte. These affinity groups include, but are not limited to deoxynucleic acid (DNA), antibody, antigen, and chelator. The signal compounds are those that can be detected using appropriate analytical methods. Preferably, but not necessarily, the signal compounds are those that produce fluorescence, chemiluminescence or electro-chemiluminescence. Examples of these signal compounds include fluorescent dyes, acridinium and its derivatives, and rare earth elements that produce fluorescence or electrochemiluminescence.

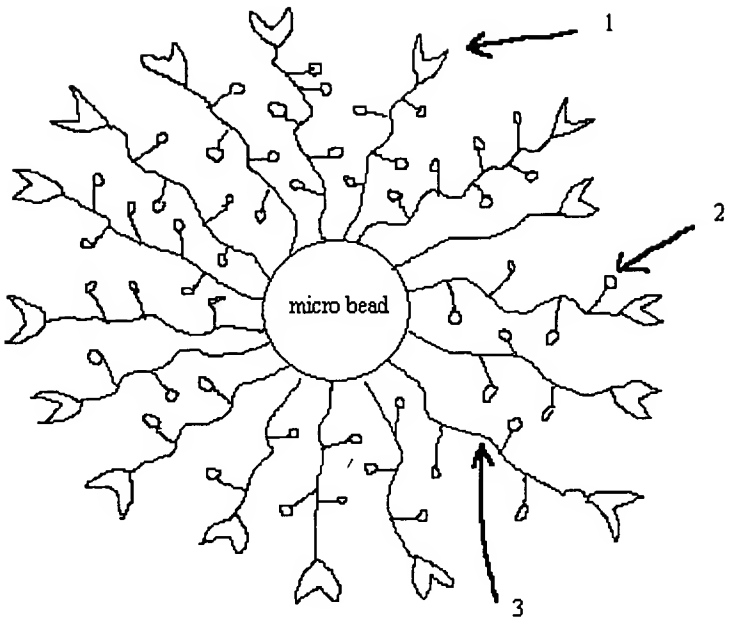

Fig 1 Layered amplifying micro sphere, 1: affinity group; 2: detecting tag (signal group) ; 3: polymer backbone

Directional labeling uses a polymer or polymers as the carrier to which both signal compounds and affinity groups can be labeled. Linear polymers (e.g., poly lysine, poly acrylic acid or modified nucleic acids) or highly branched macromolecules (such as dendrimers) can be used as the polymer backbone. They could be either natural or synthetic. One end of the polymer is attached to the microsphere whereas another end is linked to one or more affinity group; the signal group such as the fluorescent or chemoilluminant groups are normally conjugated to the polymer backbone or side chain in between the two termini. The linker between the signal groups and the polymer can also contain one or more cleavable bonds that can be cleaved using certain chemical (such as by adding a strong acid) or physical means (such as UV irradiation).

Preferably, but not necessarily, linkage between the polymers and particles and between polymer and affinity or signal groups involves covalent bonds. In certain situation, noncovalent linkage may provide a convenient alternative. For example, affinity group may be linked to the polymer via biotin-avidin binding, as illustrated in Figure 2. In this case, microparticles, which are labeled with biotin or avidin, are not analyte specific. Analyte-specific affinity group such as antibody is labeled with avidin or biotin and then bound to microparticles labeled with biotin or avidin, respectively.

Alternatively, the signal group and the affinity group can be coupled together to form a complex and the complex is coupled to the polymer or the surface of the micro particles without using any polymer.

In order to ensure that the affinity groups are located on the outermost surface of the microparticles, which provides faster and more efficient binding to the analyte, an hydrophilic group such as a PEG fragment can be attached to a position near the outer terminus of the polymer.

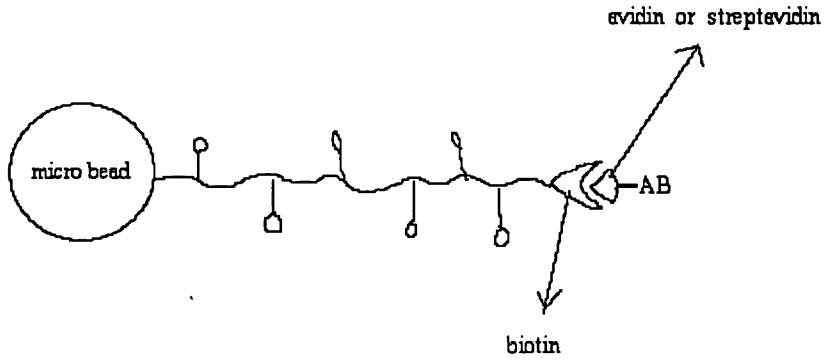

Fig 2. A universal microsphere frame for the layered amplifying micro particle, AB is antibody

The above design in fig 2. illustrates a versatile frame for this layered amplifying micro particle, using a micro particle having a self-assembly linker to couple the polymer with the affinity group (in the example here the linker is a biotin-streptavidin complex, the affinity group is a antibody AB), only different *affinity group - self-assembly linker* is need to be made for different application targeting different analytes.

It is within the scope of this invention that the affinity group is linked to microparticles through the signal group itself, as illustrated in Figure 3A. This method of labeling also gives rise to directional labeling. It is also within the scope of this invention that both the affinity group and signal group are directly labeled onto microparticles, but the linker for affinity group is sufficiently longer than that for signal group as shown in Figure 3B. The figure is a schematic view, the single S and A represents multiple S and A groups on the sphere MP.

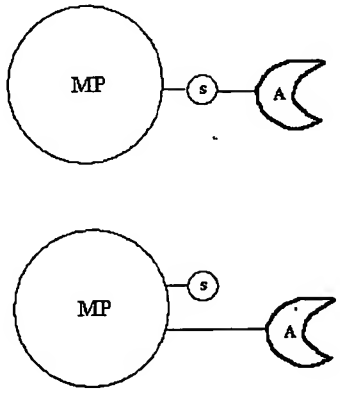

Figure 3A and 3B. (MP: microparticle; S: signal molecules; A: affinity groups)